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BACTERIOLOGICAL AND MOLECULAR STUDIES ON *E. COLI* ISOLATED FROM BROILER CHICKENS

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ABSTRACT

Avian colibacillosis is one of the most important diseases of chickens, resulting in high economic losses as well as high morbidity and mortality. In the current study, the prevalence of avian colibacillosis was studied in 200 broiler chickens obtained from different farms in Beni-Suef and El-Fayoum Governorates. A total of 200 pooling samples were collected aseptically from heart blood as well as the affected internal organs including airsacs, pericardial sac, liver, lung and ascetic fluids of slaughtered diseased and freshly dead broiler chickens. Bacteriological examination of the collected samples showed that a total of 58 *E. coli* isolates were recovered with a prevalence of 29%. The results of *in-vitro* antimicrobial susceptibility tests revealed that *E. coli* isolates showed high sensitivity to colistin sulphate only (72.4%). On the other hand, high resistances were recorded to all other antimicrobials including cefotaxime sodium and florophenicol (96.6% for each), apramycin, ciprofloxacin and gentamicin (91.4% for each), enrofloxacin and lincomycin (91.4% for each), streptomycin (89.7%), sulphamethoxazol-trimethoprim and doxycycline HCl (77.6% for each) and spiramycin (75.9%). Moreover, all *E. coli* isolates were MDR (100%). PCR was applied on 10 *E. coli* MDR isolates for detection of 7 genes; 4 resistance-associated genes (*bla*$_{TEM}$, *tet*A, *sul1* and *dfr*A) as well as 3 virulence-associated genes (*tsh*, *iss*, *iut*A). The results revealed that 90% of isolates had at least 4 virulence genes while only10% had not any gene. The gene *bla*$_{TEM}$ was the most prevalent (90%) followed by *iut*A and *sul*1 (70% for each) then, *iss* and *tet*A (60% for each). Meanwhile *dfrA* and *tsh* genes were represent in 40% and 30% of isolates, respectively.

Keywords: Broiler chickens, *E. coli*, antimicrobial susceptibility**,** multidrug resistance (MDR), resistance genes, virulence genes.

INTRODUCTION

Control of infectious diseases causing high economic loses in poultry industry is considered one of the major problems in the poultry farms (McKissick, 2006).

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Colibacillosis is caused by avian pathogenic *E. coli* (APEC) and it is considered a bacterial infection of great concern in the poultry industry. It is a widespread disease causing severe economic losses in the aviculture worldwide (Barnes *et al*., 2008). It indicates localized or systemic infections of APEC including many forms as colisepticemia, coligranuloma, airsacculitis/chronic respiratory disease (CRD), swollen-head syndrome, peritonitis/ascites, enteritis, cellulitis,

salpingitis, omphalitis/yolk sac infection, panophthalmitis, synovitis, pericarditis, and osteomyelitis (Yue *et al*., 2018). Chickens of all ages are susceptible to colibacillosis but more common in young birds which are severely affected (Barnes *et al*., 2003). Colisepticemia is the most common form of colibacillosis which is responsible for high economic losses in aviculture in many parts worldwide (Saif, 2003).

E. coli is of the most important and frequently encountered avian bacterial pathogens causing a wide range of disease syndrome in birds which cause up to 30% of poultry mortality (Radwan *et al.*, 2020). *E. coli* strains were classified by Russo and Johnson (2000) into 3 major groups including intestinal pathogenic strains, commensal strains and extra intestinal pathogenic *E. coli* (ExPEC) strains.

Although *E. coli* infections have been costly to the poultry industry, the exact virulence mechanisms used by these organisms to cause disease in birds remain interesting point of research. The presence of several virulence genes has been positively linked to the pathogenicity of APEC strains (Ewers *et al*., 2005). The establishment of PCR assays was to facilitate detection of the frequency with which the various virulence-associated genes occur in the resident APEC population; subsequently the isolates identified as the most highly pathogenic *E. coli* by PCR technique are used as the basis for the production of a powerful vaccine to be used against APEC infections. By researching the chain of infection, new and effective controls can be put in place to prevent the rapid spread of APEC (Ewers *et al*., 2004).

Since the introduction of antibiotics, there has been tremendous increase in the resistance in diverse bacterial pathogens (El-Seedy *et al*., 2019). Antimicrobial resistance of *E. coli* is a very important public health concern and of concern to poultry veterinarians (Radwan *et al*., 2020). *In-vitro* antimicrobial sensitivity testing of veterinary pathogens provides valuable guidance to the veterinarian in the choice of appropriate drug treatment (Radwan *et al*., 2016). Moreover, it is very useful to detect the multidrug resistant (MDR) isolates. Therefore, the appropriate antibiotic should be selected on the basis of its sensitivity that could be detected by laboratory examination. Resistance of *E. coli* species to antimicrobials is widespread and of concern to poultry veterinarians. This increasing resistance has received considerable attention in Egypt and worldwide. Plasmids are the major vector in the spreading of resistance genes through bacterial population (Radwan *et al*., 2020). There is a wide variety of MDR *E. coli*, and PCR can be used to detect antimicrobial resistance genes in *E. coli* isolates.

The purpose of this study was designed to perform bacteriological and molecular characterization of pathogenic *E. coli* isolated from broiler chickens.

MATERIALS AND METHODS

2.1. Chickens

A total of 200 diseased Hubbard and Ross broiler chickens of different ages (2-5 weeks) obtained from different farms in Beni-Suef and El-Fayoum Governorates were subjected to the present study during the period from January 2017 up to December 2017. These chickens were subjected to clinical and postmortem examinations. The diseased chickens had multiple internal lesions including airsacculitis, pericarditis, hepatitis and ascites.

2.2. Samples

A total of 200 pooling samples were collected aseptically from diseased broiler. Pooling swab samples were collected aseptically from heart blood as well as the lesions in the internal organs including airsacs, pericardial sac, liver, lung and

ascetic fluids of slaughtered diseased and freshly dead chickens.

2.3. Bacteriological examination

The collected samples were aseptically inoculated into MacConkey's broth and incubated aerobically at 37˚C for 24 hrs. Then a loopful of the broth culture was streaked onto tryptone soya agar and MacConkey's agar and incubated aerobically at 37˚C for 24-48hr. The lactose fermenting (pink) colonies were inoculated onto eosin methylene blue agar medium and incubated at 37˚C for 18-24 hrs and confirmed as *E. coli* morphologically and biochemically using the standard biochemical tests described by Collee *et al*. (1996) and Quinn *et al*. (2002) using the following tests; oxidase, catalase, indole, methyl red, Voges Proskauer, citrate utilization, urease, H_2S production on TSI and nitrate reduction as well as sugar fermentation. Other non-biochemical tests including motility test in semisolid agar medium and haemolysis on blood agar were applied.

2.4. Serogrouping of *E. coli* isolates

E. coli serogroups were identified serologically by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera according to Quinn *et al.* (2002).

2.5. Antimicrobial susceptibility testing

All *E. coli* isolates were tested for their antimicrobial susceptibility to 14 different antimicrobial discs including; apramycin (15µg), ciprofloxacin (15µg), cefotaxime sodium (30µg), colistin sulphate (10µg), sulphamethoxazol-trimethoprim (25µg), doxycycline HCl (30µg), enrofloxacin (5µg), lincomycin (10µg), spectinomycin (100µg), fosfomycin (300µg), gentamycin (10µg), florophenicol (30µg), streptomycin (10µg) and spiramycin (100µg) (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility testing was applied using disc diffusion method on Muller Hinton agar according to CLSI (2016). The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the CLSI (2016). Resistance to three/or more antimicrobials of different categories was taken as multidrug resistance (MDR) according to Chandran *et al*. (2008).

2.6. Polymerase chain reaction (PCR) for *E. coli* isolates

PCR was applied on 10 MDR *E. coli* isolates for detection of 7 genes; 4 resistance-associated genes (*bla*$_{TEM}$, *tet*A, *sul1* and *dfr*A) as well as 3 virulence-associated genes (*tsh*, *iss*, *iut*A). DNA extraction, primers sequences, and amplified products for the targeted genes for *E. coli* isolates were illustrated in table (1). The temperature and time conditions of the primers during PCR were shown in table (2).

Table 1: Primers of resistance and virulence genes used in PCR.

Primer			Primer sequence (5'-3')	Amplified product	Reference
Resistance genes	*bla*$_{TEM}$	*F*	*ATCAGCAATAAACCAGC*	516 bp	**Colom *et al*. (2003)**
		R	*CCCCGAAGAACGTTTTC*		
	*tet*A	*F*	*GGTTCACTCGAACGACGTCA*	576 bp	**Randall *et al.* (2004)**
		R	*CTGTCCGACAAGTTGCATGA*		
	*sul*1	*F*	*CGGCGTGGGCTACCTGAACG*	443 bp	**Sabarinath *et al*. (2011)**
		R	*GCCGATCGCGTGAAGTTCCG*		
	*dfr*A	*F*	*AGCATTACCCAACCGAAAGT*	817 bp	**Huovinen *et al.* (1995)**
		R	*TGTCAGCAAGATAGCCAGAT*		
Virulence genes	*iut*A	*F*	*GGCTGGACATGGGAACTGG*	300 bp	**Yaguchi *et al*. (2007)**
		R	*CGTCGGGAACGGGTAGAATCG*		
	iss	*F*	*ATGTTATTTTCTGCCGCTCTG*	266 bp	
		R	*CTATTGTGAGCAATATACCC*		
	tsh	*F*	*GGTGGTGCACTGGAGTGG*	620 bp	**Delicato *et al.* (2003)**
		R	*AGTCCAGCGTGATAGTGG*		

Table 2: Cycling conditions of the different primers during PCR.

Gene	Primary denaturing	secondary denaturing	Annealing	Extension	No. of cycles	Final extension
- ***bla*$_{TEM}$**	94C/10min	94C/45sec.	54C/45sec.	72C/45sec.	35cycles	72C/10min.
- ***tet*A**	94C/5min.	94C/45sec.	50C/45sec.	72C/45sec.	35cycles	72C/10min.
- ***sul*1**	94C/5min.	94C/45sec.	60C/45sec.	72C/45sec.	35cycles	72C/10min.
- ***dfr*A**	94C/10min	94C/1min.	50C/1min.	72C/1min.	35cycles	72C/10min.
- ***iutA***	94C/5min.	94C/30sec.	63C/30sec.	72C/30sec.	35cycles	72C/7min.
- ***iss***	94C/5min.	94C/30sec.	54C/30sec.	72C/30sec.	35cycles	72C/7min.
- ***tsh***	94C/5min.	94C/45sec.	54C/45sec.	72C/30sec.	35cycles	72C/10min.

RESULTS

3.1. Prevalence of *E. coli* isolation in the diseased broiler chickens

Out of 200 diseased broiler chickens, 58 *E. coli* isolates were recovered with an overall prevalence rate of 29%.

3.2. Serogrouping of *E. coli* isolates

Out of 58 *E. coli* isolates, 7 O-serogroups were obtained. The serogroups O_{125} was the most prevalent represented 18 isolates (31%) followed by serogroups O_{158} (*n=14*; 24.1%) and O_{55} (*n=8*; 13.8%). Then, the serogroup O_{78} (*n=6*; 10.3%). Afterthat, serogroups O_1 (*n=4*; 6.9%) and finally serogroups O_{15} and O_8 (*n=2*; 3.4% for each). Moreover, there were 4 isolates (6.9%) were untyped with the available antisera (Table 3).

Table 3: *E. coli* serogroups recovered from broiler chickens.

	E. coli Serogroup	No. of tested isolates	%
-	**O_{125}**	**18**	**31**
-	**O_{158}**	**14**	**24.1**
-	**O_{55}**	**8**	**13.8**
-	**O_{78}**	**6**	**10.3**
-	**O_1**	**4**	**6.9**
-	**O_{15}**	**2**	**3.4**
-	**O_8**	**2**	**3.4**
Total serotyped isolates		**54**	**93.1**
-	**Untyped**	**4**	**6.9**
	Overall total	**58**	**100**

%: was calculated according to the overall total number (No.) of tested isolates (*n=58*).

3.3. Antimicrobial susceptibility testing

Results of *in-vitro* susceptibility testing showed that *E. coli* isolates were highly resistant to most of the tested antimicrobials. The highest resistance was recorded against cefotaxime sodium and florophenicol (96.6% for each) followed by apramycin, ciprofloxacin and gentamicin (93.1% for each). Then, enrofloxacin and lincomycin (91.4% for each), streptomycin (89.7%), sulphamethoxazol-trimethoprim and doxycycline HCl (77.6% for each) and spiramycin (75.9%). Finally, fosfomycin (55.2%) and spectinomycin (51.7%). On the other hand, they were highly sensitive to colistin sulphate only (72.4%). MDR was detected in all the tested isolates (100%) (Table 4).

Table 4: Results of antimicrobial susceptibility testing of E. coli recovered from diseased broiler chickens.

Antimicrobial disc	Disc content (µg)	*E. coli* (n=58)					
		R		I		S	
		No	%	No	%	No	%
Apramycin	**15**	54	93.1	4	6.9	0	0
Ciprofloxacin	**5**	54	93.1	2	3.4	2	3.4
Cefotaxime sodium	**30**	56	96.6	2	3.4	0	0
Colistin sulphate	**10**	12	20.7	4	6.9	42	72.4
Sulfamethoxazole-trimethoprem	**25**	45	77.6	7	12.1	6	10.3
Doxycycline HCl	**30**	45	77.6	9	15.5	4	6.9
Enrofloxacin	**5**	53	91.4	3	5.2	2	3.4
Lincomycin	**10**	53	91.4	1	1.7	4	6.9
Spectinomycin	**100**	30	51.7	7	12.1	21	36.2
Fosfomycin	**300**	32	55.2	3	5.2	23	39.7
Gentamycin	**10**	54	93.1	4	6.9	0	0
Florphenicol	**30**	56	96.6	0	0	2	3.4
Streptomycin	**10**	52	89.7	3	5.2	3	5.2
Spiramycin	**100**	44	75.9	3	5.2	11	19

% was calculated according to the number of the tested isolates (n=80).

2.4. Polymerase chain reaction (PCR) analyses of *E. coli* isolates

Regarding the resistance-associated genes, PCR results revealed that *bla*$_{TEM}$ was the most prevalent gene present in 9 *E. coli* isolates (90%) followed by *sul*1 (*n=7*; 70%), *tet*A (*n=6*; 60%) and *dfr*A (*n=4*; 40%) (Table 5 and Figs. 1-4).

On the other hand, PCR results of virulence-associated genes revealed that *iut*A was the most prevalent gene present in 7 *E. coli* isolates (70%) followed by *iss* (*n=6*; 60%) and *tsh* (*n=3*; 30%) (Table 5 and Figs. 5-7).

Collectively, 9 isolates (90%) had at least 4 genes while only one isolate (10%) had not any gene.

Table 4: Prevalence and distribution of virulence and resistance- associated genes in the examined *E. coli* isolates.

Gene / Sample	Virulence genes			Resistance genes			
	tsh	*iss*	*iut*A	*bla*$_{TEM}$	*dfr*A	*sul*1	*tetA*
1	-	+	-	+	+	+	+
2	-	-	-	-	-	-	-
3	+	-	+	+	-	+	-
4	-	+	+	+	-	-	+
5	-	+	+	+	+	+	+
6	-	-	+	+	-	+	+
7	-	+	-	+	+	+	+
8	-	-	+	+	-	+	+
9	+	+	+	+	+	+	-
10	+	+	+	+	-	-	-
Total	**3**	**6**	**7**	**9**	**4**	**7**	**6**
%	**30**	**60**	**70**	**90**	**40**	**70**	**60**

%: was calculated according to the number (No.) of the tested isolates (*n=10*).

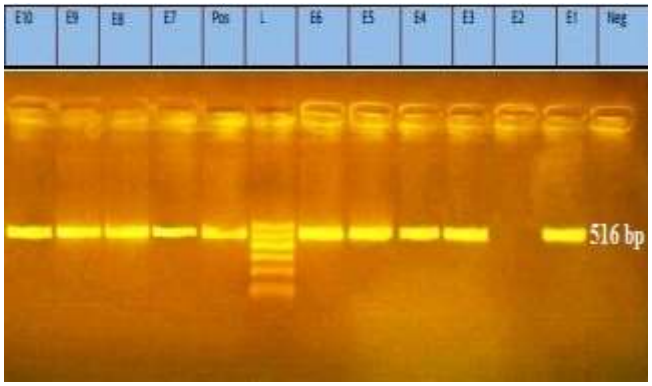

Fig. (1): PCR amplification of the 516 bp fragment of *bla*$_{TEM}$ resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

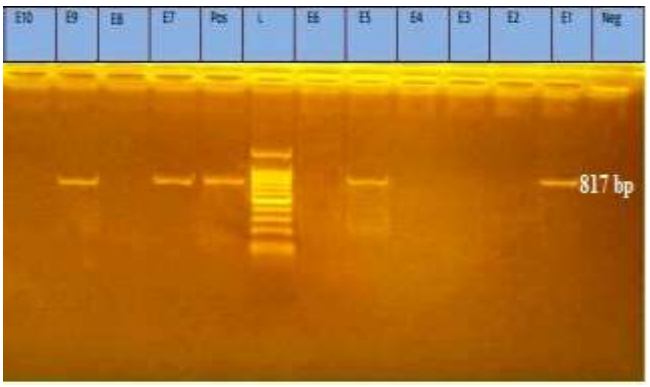

Fig. (2): PCR amplification of the 817 bp fragment of *dfr*A resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

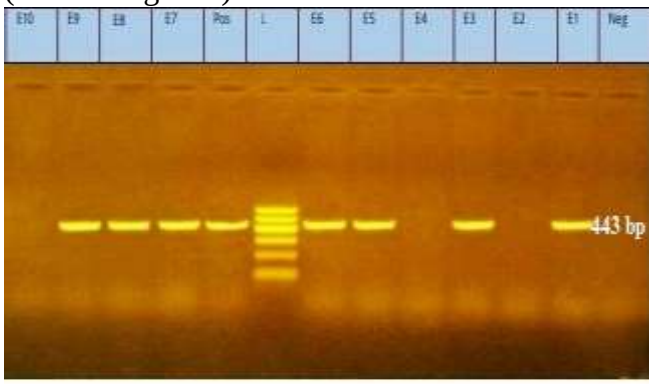

Fig. (3): PCR amplification of the 443 bp fragment of *sul*1 resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

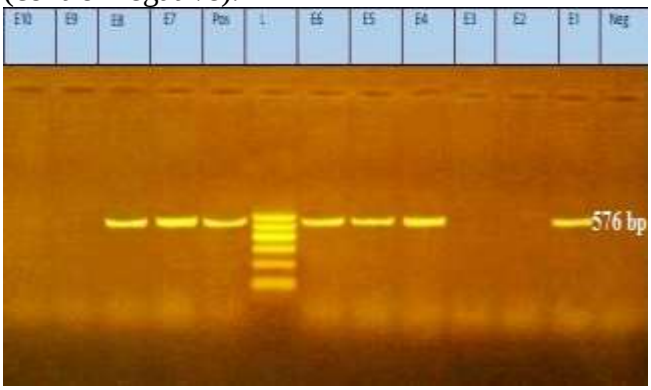

Fig. (4): PCR amplification of the 576 bp fragment of *tet*A resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

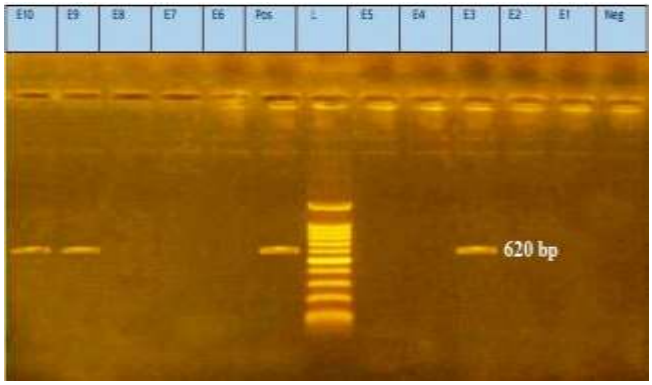

Fig. (5): PCR amplification of the 620 bp fragment of *tsh* virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

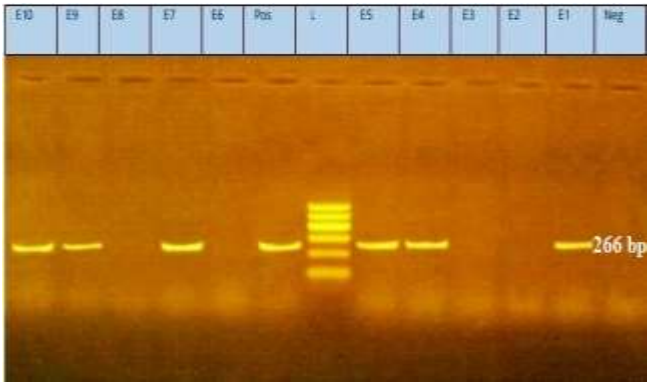

Fig. (6): PCR amplification of the 266 bp fragment of *iss* virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

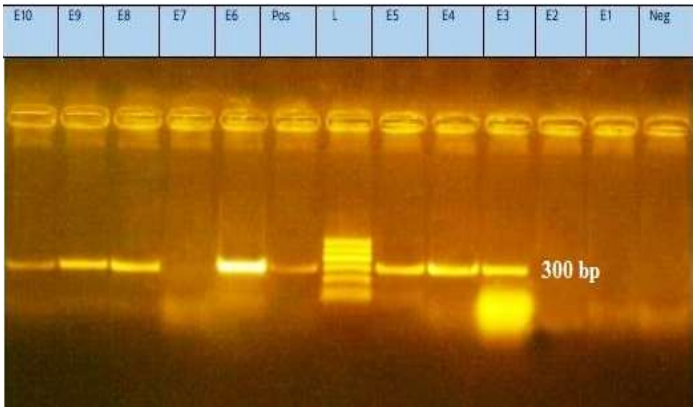

Fig. (7): PCR amplification of the 300 bp fragment of *iut*A virulence gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

DISCUSSION

Avian colibacillosis is one of the most important diseases of chickens, resulting in significant economic losses as well as high morbidity and mortality among baby chicks, broilers and layers (Ewers *et al.,* 2004, Paixão *et al.,* 2016). This syndrome is characterized by acute septicemia with considerable death rates as well as sub-acute forms characterized by multiple organ lesions including airsacculitis and associated pericarditis, perihepatitis and peritonitis (Huja *et al.,* 2015 and Younis *et al.,* 2017). Stress seemed to cause invasion of APEC from intestine into blood stream and spreads into various internal organs and typically causes pericarditis, perihepatitis, peritonitis, salpingitis and other extra-intestinal diseases (Leinter and Heller, 1992).

In the current study, the prevalence of avian colibacillosis was studied in broiler chickens. The results revealed that the prevalence of *E. coli* in the diseased broiler chickens was 29% as 58 *E. coli* isolates were recovered from 200 diseased broiler chickens. In Egypt, these results were nearly similar to that obtained by Radwan *et al.* (2020) who recorded a prevalence of 26.7% in diseased broiler chickens. Other nearly similar results were recorded (Ammar *et al.*, 2011; 24%, Abd El Tawab *et al.*, 2014; 24.7%, El-Seedy *et al.*, 2019; 23%). This observation was also was slightly lower than those previously described by Younis *et al.* (2017); 36.5%**,** and Qurani (2019); 33%. Higher results were obtained by Roshdy *et al*. (2012); 43.1%, and Radwan *et al.* (2014); 41.5%, Meanwhile, much higher prevalences were recorded including El-Sukhon *et al*. (2002); 88.2%, Abd El-Latif (2004); 78.7%, Abd El Aziz *et al.* (2007); 90%, and Radwan *et al.* (2016); 56%.

These variations in the *E. coli* prevalence in broilers may be attributed to the difference in strains pathogenicity and virulence beside the severity of the cases as well as the immunological status of the host (Radwan *et al.,* 2020). Moreover, Ashraf *et al.* (2015) attributed the variation in *E. coli* prevalence to the difference in the seasons as they recorded higher prevalence in winter (60.9%) than that in summer (41%). This difference might be due to the lower environmental and hygienic conditions in poultry farms in winter such as overcrowding, bad ventilation and higher ammonia level in air. Also, it might be due to higher percentage of *E. coli* in feed, water, litter and air in winter than in summer. Also, Abd El Tawab *et al.* (2015) recorded prevalences of *E. coli* in samples from apparently healthy, diseased and freshly dead broiler chickens in winter (15.7%, 37.1% and 55%, respectively) and summer seasons (15.8%, 17.5% and 18.7%, respectively). Stress may cause invasion of pathogenic *E. coli* from intestine into blood stream and spreads into different visceral organs causing peritonitis, perihepatitis,

pericarditis, salpingitis and other extra-intestinal diseases (Leinter and Heller, 1992).

Results of serogrouping of *E. coli* isolates were illustrated in table (3). Seven O-serogroups were obtained. Serogroups O_{125} was the most prevalent represented as 31% followed by serogroups O_{158}, O_{55}, O_{78} as 24.1%, 13.8% and 10.3%, respectively. Then, O_1 represented as 6.9% and finally serogroups O_{15} and O_8 (3.4%, for each). Moreover, 6.9% of isolates were untyped with the available antisera. The distribution of O antigens was nearly similar to that reported in previous studies (Messier *et al.*, 1993; Gomis *et al.*, 2001 and Schouler *et al*., 2012) who recovered nearly the same serogroups; beside other serogroups. On the contrary they differed from those obtained by Tana *et al.* (2013) who recovered 8 different serogroups *E. coli* including O_2, O_8, O_{15}, O_{73}, O_{86}, O_{102}, O_{115} and O_{139}, and Wang *et al.* (2010) who recovered 8 serogroups; O_{65}, O_{78}, O_8, O_{120}, O_2, O_{92}, O_{108}, and O_{26}.

Antimicrobial therapy is considered one of the worldwide primary controls for reduction of both morbidity and mortality associated with avian colibacillosis therefore reducing their great losses in the poultry industry (Radwan *et al*., 2016). Although antimicrobials are considered valuable tools prevent and treat infectious bacterial diseases and as growth promoters at sub-therapeutic levels in feeds to maintain health and productivity of birds, its use in livestock production has been implicated as a risk factor in the development and spreading of antibiotic resistance (Gosh and LaPara, 2007). Increasing of antimicrobial resistance is a very important public health concern, and the emergence and spread of antimicrobial resistance is a complex problem driven by numerous interconnected factors. *In-vitro* antimicrobial sensitivity testing of veterinary pathogens provides valuable guidance to the veterinarian in the choice of appropriate drug treatment (Radwan *et al*., 2016). Moreover, it is very useful to detect the MDR isolates. Therefore, the appropriate antibiotic should be selected on the basis of its sensitivity that could be detected by laboratory examination.

In the current work, *E. coli* isolates were tested for their susceptibility to 14 different antimicrobial drugs to detect the drug of choice for treatment as well as to detect MDR isolates for further analyses of the isolates. The results of *in-vitro* antimicrobial susceptibility tests for *E. coli* isolates were demonstrated in table (4). *E. coli* isolates showed high sensitivity to colistin sulphate only (72.4%). On the other hand, high resistances were recorded against most of the tested antimicrobials especially cefotaxime sodium, florophenicol, apramycin, ciprofloxacin and gentamicin, enrofloxacin, lincomycin and streptomycin (89.7-96.6%) as well as sulphamethoxazol-trimethoprim, doxycycline and spiramycin (75.9-77.6%). MDR was detected in all the tested isolates (100%).

Regarding the result of colistin sulphate susceptibility, it was supported by several previous reports in Egypt and worldwide. In Egypt, the current result was the same with those obtained by Radwan *et al.* (2020) who applied the *in-vitro* susceptibility on 80 *E. coli* isolates against 11 antimicrobials and found that 70% of strains were sensitive to colistin meanwhile El-Seedy *et al.* (2019) found that colistin had the highest sensitivity (63.6%).

Regarding the increasing incidences of antibiotic-resistance of *E. coli* isolates in such study; these findings were coincided with those recorded by many authors in Egypt (Abd El Tawab *et al*. 2014& 2015; Radwan *et al.*, 2014, 2016 and 2018& 2020; Awad *et al*., 2016; El-Shazly *et al.*, 2017; Amer *et al*., 2018; El-Seedy *et al*., 2019 and Qurani, 2019). Therefore, no single antimicrobial drug was effective by 100% against *E. coli* isolates, which might be due to development of resistance due to indiscriminate use of antibiotics (Sharada *et al.*, 2001).

Moreover, in the current study, MDR was detected in all *E. coli* isolates (100%). Such results agreed also with several previous reports in Egypt and all over the world. In Egypt, Amer *et al*. (2018); Qurani (2019) and Radwan *et al.* (2020) found that all *E. coli* isolates were MDR. Meanwhile, Radwan *et al.* (2014) recorded MDR in 90.4% of isolates.

The spread of MDR bacteria has been recognized as an increasing problem in both medical and veterinary fields, and mobile DNA elements such as plasmids, integrons and transposons favor the proliferation of resistance genes in the bacteria (Speer *et al*., 1992 and Liebert *et al*., 1999). Antimicrobial resistance of *E. coli* species is widespread and of concern to poultry veterinarians. This increasing resistance has received considerable attention in Egypt and worldwide. Plasmids are the major vector in the spreading of resistance genes through bacterial population (Radwan *et al*., 2016). The R-plasmids have been extensively studied in view of the prevalence of MDR (O'Brien *et al.,* 1982). Several virulence and resistance associated genes were reported on plasmids of *E. coli* recovered from diseased poultry (Kelly *et al.*, 2009). There is a wide variety of MDR *E. coli* and PCR can be used to detect antimicrobial resistance genes in *E. coli* isolates.

In the current work, PCR was applied on 10 MDR *E. coli* isolates to detect the 3 resistance-associated genes including plasmid-mediated genes for resistance to β-lactamase (*bla*$_{\text{TEM}}$), sulfonamides (*sul*1), tetracycline (*tet*A) and trimethoprim (*dfr*A). The results represented in table (5) and figs. (1-4) showed that *bla*$_{\text{TEM}}$ was the most prevalent gene represented in 90% of the tested isolates, followed by *sul*1 (70%), *tet*A (60%) and *dfr*A (40%). These results were the same of those reported by Radwan *et al.,* (2016) who recorded *bla*$_{TEM}$ gene as the most prevalent found in all isolates (100%) followed by *sul*1(92.9%), *tet*A (35.7%) and *dfr*A (21.4%). Also, these results run parallel to those obtained by Adelowo *et al.* (2014); *bla-TEM* (85%), *sul2* (67%) and *tetA* (21%). Also, Momtaz *et al.* (2012) detected the distribution of antibiotic-resistant genes in *E. coli* isolates from slaughtered commercial chickens as follow *tetA* (52.63%), *sul1* (47.36%) and *dfrA1* (36.84%). Moreover, Glenn *et al.* (2012) detected the four genes in *E. coli* isolates from broilers while Ahmed *et al.,* (2009) detected *bla*$_{\text{TEM}}$ and *dfr*A genes in *E. coli* isolates recovered from retail chicken meat.

Not all APECs are equally virulent. Highly pathogenic APECs generally cause primary infections while less pathogenic strains only cause poultry disease under severe stress conditions including other diseases or environmental stresses leading to compromised host immunity then secondary infections can occur (Radwan *et al.*, 2020). Virulence in APEC is caused by virulence genes present either in the chromosome or on the plasmids (Dozois *et al*., 2003). Multiple varieties of virulence-associated genes exist and are associated with colibacillosis and several virulence-encoded genes were harbored on plasmids of APEC (Kelly *et al.*, 2009). The highly virulent *E. coli* strains carried at least 4 virulence encoded genes on their largest plasmids (Tivendale *et al*., 2004).

The virulence mechanisms of APEC were summarized in three steps; adhesion, followed by a multiplication in the host's tissues and finally the evasion of its defense systems (Radwan *et al.*, 2016). APEC strains may produce temperature sensitive haemagglutinin (*tsh*) which is considered one of the adhesion factors encoded by a *tsh* gene. This gene is located in ColV plasmids which are frequently found in highly pathogenic avian *E. coli* and rarely detected in commensal *E. coli* (Delicato *et al.*, 2003). Because the association of the *tsh* gene with APEC pathogenicity, Ewers *et al*. (2004) proposed its utilization as a molecular marker to detect APEC strains.

Moreover, APEC possesses a number of other virulence factors that enable them to survive in the extra-intestinal tissues of the host. Serum resistance was found to be an important virulence determinant for *E. coli* in chickens and turkeys (Delicato *et al*., 2003). The *iss* (increased serum survival) gene is associated with serum resistance and it is significantly more often present in APEC than in commensal *E. coli* (Vandekerchove, 2004). The *iss* gene has been identified as a distinguishing trait of avian but not of human (Johnson *et al.*, 2008) and its occurrence in conjugative Col V plasmid can suggest the relationship of *iss* factor to the APEC pathogenicity.

APEC strains survive and growth in environments with low iron availability, mainly inside the host, because the expression of iron acquisition systems; like aerobactin, which are associated with virulence in chickens (Vandekerchove, 2004). Most APEC produce aerobactin, while this siderophore is absent in most commensal *E. coli* (Delicato *et al*., 2003). The gene encoding aerobactin receptor is called *iutA* (iron uptake system).

Various studies have highlighted the ability of using some virulence associated genes for identifying APEC strains. They have attempted to detect a common scheme for identification allowing better identification of APEC strains than serotyping. These methods are mainly based on genotyping using PCR methods for the detection of virulence genes carried on colicin V (ColV) plasmids (Radwan *et al*., 2014). The genotyping methods allow more identification of APEC isolates with higher reliability than the classical serotyping methods used in veterinary labs (Schouler *et al.*, 2012). More than 90% of the total APEC examined possessed *iss, tsh, iut*A, *hly*F and *omp*T genes (Radwan *et al*., 2020).

In the current study, PCR was applied on 10 MDR *E. coli* isolates to detect 3 virulence-associated genes including temperature sensitive haemagglutinin gene (*tsh*), increased serum survival gene (*iss*) and iron transport encoding gene (*iut*A). The results represented in tables (5) and figs. (5-7) showed that *iut*A was the most prevalent represented in 70% of the tested isolates followed by *iss* (60%) and *tsh* (30%). These results were nearly similar same of those reported by Radwan *et al.* (2016) who recorded *iutA* gene as the most prevalent found in 78.6% of isolates followed by *i*ss (71.4%) and *tsh* represented as 28.6%. Also, these results were nearly similar to those of Campos *et al.* (2005) who demonstrated that the *tsh* gene was found among 25% APEC strains isolated from chickens with colisepticemia as well as Maurer *et al*. (1998) who detected the *tsh* gene among 46% of the studied APEC strains and in none of the commensal isolates. On the other hand they were opposite to those of Radwan *et al.* (2014) who reported the prevalence of *iss* and *iutA* genes as 75% and 5%, respectively, Moon *et al.* (2006); *tsh* (55%), *iutA* (50%), *iss* (41%), and Kwon *et al*. (2008) who reported that 100% were carrying *iss* gene while 94%were carrying *tsh* gene. In addition, many authors detected the three genes (Delicato *et al.*, 2003; Tivendale *et al.*, 2004; Ewers *et al*., 2005; Johnson *et al*., 2008; Kobayashi *et al.*, 2011 and Radwan *et al.*, 2016).

CONCLUSION

Colibacillosis is one of the most important diseases of chickens, resulting in significant economic losses as well as high morbidity and mortality. Presence of multidrug resistance pathogens occurred due to the misuse of the antibiotics and is considered a great problem. The prevalence of *E. coli* in the diseased broiler chickens was 29%. Among 7 O-serogroups were obtained, O_{125} was the most prevalent represented as 31%. The results of *in-vitro* antimicrobial susceptibility tests for *E. coli* isolates showed high sensitivity to colistin sulphate only. On the other hand, high resistances were recorded against most of the tested

antimicrobials. MDR was detected in all the tested isolates (100%). The results of PCR in revealed that 90% of isolates had at least 4 virulence genes while only10% had not any gene. The gene *bla*$_{TEM}$ was the most prevalent (90%) followed by *iut*A and *sul*1 (70% for each) then, *iss* and *tet*A (60% for each). Meanwhile *dfrA* and *tsh* genes were represent in 40% and 30% of isolates, respectively.

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دراسات بكتريولوجية وجزيئية عن الايشيريشيا كولاي المعزولة من بداري التسمين

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تعتبر الاصابة بالميكروب القولوني أحد أهم الأمراض التي تصيب الدجاج ، مما يؤدي إلى خسائر اقتصادية عالية ، فضلاً عن ارتفاع معدلات انتشار المرض والنفوق. في الدراسة الحالية تمت دراسة انتشار داء قولونيات الطيور في 200 دجاجة تسمين من مزارع مختلفة في محافظتي بني سويف والفيوم. تم جمع 200 عينة مجمعة معقمة من دم القلب وكذلك الأعضاء الداخلية المصابة بما في ذلك الأكياس الهوائية غشاء التامور والكبد والرئة وسوائل الاستسقاء من دجاج التسمين المذبوحة والميتة حديثًا. أظهر الفحص البكتريولوجي للعينات التي تم جمعها أنه تم عزل ما مجموعه 58 عزلة من الإشريشيا كولاي بنسبة انتشار بلغت 29٪. أظهرت نتائج اختبارات الحساسية لمضادات الميكروبات في المختبر أن عزلات الإشريشيا كولاي أظهرت حساسية عالية لسلفات الكوليستين فقط (72.4٪). من ناحية أخرى ، تم تسجيل مقاومة عالية لجميع مضادات الميكروبات الأخرى بما في ذلك سيفوتاكسيم الصوديوم والفلوروفينيكول (96.6٪ لكل منهما) والأبراميسين والسيبروفلوكساسين والجنتاميسين (91.4٪ لكل منهما) والإنروفلوكساسين واللينكومايسين (91.4٪ لكل منهما) والستربتومايسين (89.7٪ لكل منهما)، سلفاميثوكسازول-تريميثوبريم ودوكسيسيكلين هيدروكلورايد (77.6٪ لكل منهما) وسبيرامايسين (75.9٪). علاوة على ذلك ، كانت جميع عزلات الإشريكية القولونية متعددة المقاومة للمضادات الميكروبية (١٠٠٪). تم تطبيق تفاعل البلمرة المتسلسل على 10 عزلات من الإشريكية القولونية متعددة المقاومة كتشاف 7 جينات. 4 جينات مرتبطة بالمقاومة (*bla*$_{TEM}$ و *tet*A و *sul*1 و *dfr*A) بالإضافة إلى 3 جينات مرتبطة بالضراوة (*iss* ، *tsh*، *iut*A). أظهرت النتائج أن 90٪ من العزلات تحتوي على 4 جينات ضراوة على الأقل بينما 10٪ فقط لا تحتوي على أي جين. كان الجين *bla*$_{TEM}$ هو الأكثر انتشارًا بنسبة 90 ٪ يليه *iut*A و *sul*1 بنسبة 70٪ لكل منهما ، ثم *iss* و *tet*A بنسبة 60 ٪ لكل منهما. بينما تم تمثيل جينات *dfr*A و *tsh* في 40٪ و 30٪ من المعزولات على التوالي.